Original Research Article

Microbial Assessment of Foods and Currencies from Street Food Vendors and Antibiotic Resistance Profile of Isolates

ABSTRACT

Aims: Street food vendors handle money and food simultaneously, creating possibilities of cross contamination. This study focused on determining the microbial loads of street vended foods and currency denominations received as change; identify and determine the antibiotics resistance profile of the isolates.

Materials and Methods: Street vended food samples and currency (10 - 500 Naira) notes from four vendors (FV) were collected. Microbial loads of food and money, biochemical (MRVP, motility carbohydrate fermentation) and antibiotic testing of isolates were performed using standard methods.

Results: TVC value ranged from (1.0×10^7 to 1.7×10^8 CFU/g); Staphylococcal (5.8×10^6 to 1.6×10^7 CFU/g), *Salmonella-Shigella* (NG to 1.2×10^7 CFU/g) and Coliform (no growth) from FV1. TVC ranged from (4.2×10^6 to 2.0×10^7 CFU/g); Staphylococcal (1.2×10^6 to 2.0×10^7 CFU/g), *Salmonella-Shigella* (1.6×10^6 to 1.1×10^7 CFU/g) and Coliform (NG to 1.5×10^5 CFU/g) from FV2. From FV3 TVC ranged from (9.3×10^6 to 4.6×10^7 CFU/g); Staphylococcal (1.9×10^6 to 5.1×10^6 CFU/g), *Salmonella-Shigella* (1.3×10^6 to 5.9×10^6 CFU/g) and Coliform (5.0×10^3 to 1.0×10^6 CFU/g). FV4 load ranged from (2.8×10^6 to 1.3×10^8 CFU/g); Staphylococcal (1.7×10^6 CFU/g to TNTC), *Salmonella-Shigella* (NG to 5.7×10^7 CFU/g) and Coliform (6.5×10^4 to 6.8×10^5 CFU/g). Eleven genera were identified from food and currencies including: *Staphylococcus, Klebsiella, E. coli* and *Enterobacter.* Organisms showed varied resistance patterns to the different antibiotics screened. About (62.5 %) of isolates were resistant to Ampicillin (10 µg) and (61.5 %) Ceftazidime (30 µg).

Conclusion: In conclusion, there are possibilities of cross contamination between food and currencies making the safety of consuming the foods a public health concern.

Keywords: Street foods; food; money; simultaneous handling; safety.

1. INTRODUCTION

Street foods are ready–to-eat foods and beverages prepared and/or sold by vendors on streets and other public places [1]. Street foods are sources of nutrition for many, at affordable prices. Some of the popular street foods sold in Nigeria include: rice, beans, akara (fried bean cake), boli (roasted plantain), roasted corn, amala (yam flour meal), garri, fufu (cassava meal), fried fish, stick or peppered meat, suya (peppered stick meat), fruits, salads and more. The street food industry plays an important role in developing countries in meeting the food demands of the urban dwellers [2]. There is a noticeable increase of food vendors in Nigeria as a result of dwindling economy and unemployment [2]. Types of vending sites encompass stalls, a variety of push-carts, roadside stands, and hawkers depending upon the ingenuity of the individual, resources available and type of food sold [3].

Trading has been part of mankind from time immemorial. Items passed from hand to hand are likely to be contaminated with disease causing microorganism especially if handled with unclean hands or kept in dirty surroundings [4]. Paper and polymer currency notes may habour various deadly pathogenic microorganisms, and this could represent a universal medium of transmission of bacteria in the environment and among humans [5].

Many of the street food vendors serve foods and collect money for the foods simultaneously and do not wash or sanitize their hands between tasks [6, 7, 8]. In spite of numerous advantages offered by street foods, there are several health hazards associated with this sector of the economy. There is a possibility that currency notes might act as vehicles for the transmission of potential pathogenic microorganisms from human and environment into foods especially during simultaneous handling of food and money by street food vendors. Money on which pathogenic microorganisms might survive represents an often over looked reservoir for enteric disease [9, 10,11].

Vendors often lack formal education and untrained in food hygiene; work under crude and unsanitary conditions and have very little knowledge about the causes of foodborne diseases [3]. Evidently, since street vended foods have been shown to have epidemiological links with illness [12, 13, 14, 15], it further suggests that street foods contribute immensely to food poisoning outbreaks.

Furthermore, the rise of street food vending and simultaneous handling of money by same vendors has created opportunities for contaminations, resulting in public health problems [16, 17]. Unsafe food is becoming an increasingly serious threat to public health in Nigeria.

Perpetual and indiscriminate administration of antimicrobial agents and antibiotics in developing countries coupled with poor hygiene habits has led to the development of multiple drug resistant microorganisms. Furthermore, the prevalence of antibiotic resistance among foodborne pathogens has increased in recent times [18]. Failure of food service workers to adequately wash and or sanitize hands between handling of money and serving of food could put consumers at risk of diarrheal diseases. Therefore, the aim of this study was to assess the microbial quality of food and currency denominations from street food vendors for possibility of cross contaminations and determine the antibiotic resistance profile of isolates.

2. MATERIAL AND METHODS

2.1 SAMPLE COLLECTION

Four types of food samples namely: Ekuru, jollof rice, pounded yam with sauce and suya were procured from food (stationary and mobile) vendors in Iwo, Osun State. Samples were collected in sterile polyethylene bags and kept in an ice pack inside fo flasks for transportation to Bowen University Food Science laboratory and isolation from samples were carried out within 24 hours of purchase. Table 1 describes the food categories. Also, the currency notes returned as change after purchase were placed in separate Ziploc bags. Both food items and the currency notes were used for analyses.

Table 1: Description of food samples obtained during research

Food categories	Composition of Food in the category
Ekuru	Uncoated bean paste, pepper sauce, oil and meat
Jollof rice	Rice, pepper sauce, oil and meat
Pounded Yam	Yam, vegetable stew, pepper sauce and meat
Suya	Steak meat, ground pepper, oil and seasoning

2.2 Microbial analysis of food

About 10 g of the food samples was homogenized in 90 mL peptone water and mixed properly. Then, 1 mL of sample was serially diluted in 0.85 % saline solution up to 10^{-5}. Using the pour plate method, 1 mL of the last dilution was plated into plate count agar; Eosin methylene blue agar (LAB); *Salmonella- Shigella* agar (SRL); Baird Parker agar; and Sabouraud dextrose agar (SDA) (Park Scientific Limited) in duplicates and incubated at 37 ^{0}C overnight while SDA plates were incubated at 28 ^{0}C for 72-96 h. All plates with less than 300 colonies were enumerated after incubation.

Microbial analysis of paper currencies

Collected currency notes from each vendor were analyzed separately. Notes were separated into denominations of polymer (10, 20, 50 Naira); 100 Naira; 200 Naira and 500 Naira. Each denomination was aseptically placed into a new Ziploc bag. About 100 mL of peptone water was added and the samples were shaken vigorously for 10 mins in order to dislodge adhering microorganisms. Further serial dilution up to 10^{-4} was performed, plating, incubation and enumeration were as previously described.

2.3 Identification of isolated microorganisms

After enumeration, colonies with distinct morphological differences like color, size and shape were randomly selected for further biochemical analyses. Isolates were purified by repeated plating and preserved on Nutrient agar slants and stored at 4 °C until characterization was carried out. Isolates were characterized using Gram reaction, motility, sugar fermentation test, MR VP test, citrate utilization test, starch hydrolysis test and catalase test [19].

2.4 Antibiotic resistance testing

A total of 13 (5 Gram positive and 8 Gram negative) isolates were randomly selected and tested for antimicrobial resistance profile. About 5 mL of nutrient broth was inoculated with a loopful of the organism and incubated at 37 °C overnight to an inoculum density equivalent to a 0.5 McFarland turbidity standard. Sterile cotton swab was moistened with each isolate and used to swab Muller-Hinton (Biomark, India) agar plates in duplicates. Then, the plates were left to dry for about 5 mins before placing the antibiotics discs (Rapid Labs, UK) on each of the plates as described by [20]. Antibiotic discs and concentrations used were: Ceftazidime (CAZ) – 30 µg; Cefuroxime (CRX) – 30 µg; Gentamicin (GEN) – 10 µg; Cefriaxone (CTR) – 30 µg; Erythromycin (ERY) – 5 µg; Cloxicillin (CXC) -5 µg; Ofloxacin (OFL) – 5 µg; Amoxycillin/ Clavulanate (AUG) -30 µg; Ciprofloxacin (CPR) – 5 µg; Nitrofurantoin (NIT) – 300 µg; and Ampicillin (AMP) – 10 µg. Zoning diameter was taken as the mean zone along the two directions on perpendicular lines using a ruler on the reverse side of the plates and measured in millimeters. In addition, zone size was expressed as susceptible/resistant based on the recommendation of National Committee for Clinical Laboratory Standards Institute (National Committee for Clinical Laboratory Standards [21].

2.5 Statistical Analysis

Data collected from microbial load were analyzed using Statistical Package for the Social Sciences (SPSS) (2011) [22]. Analysis of Variance (ANOVA) was used to evaluate significant differences and separation of the mean values was carried out using Duncan Multiple Range Test at ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1. Microbial loads of food and currency denomination from food vendors

The results of microbial loads of the food samples and currency denominations (Naira) received as change after purchase are presented in Tables 2. Overall, all of the samples analyzed in this study had microbial contamination. Ekuru had ($1.7x10^8$ CFU/g); ($1.3x10^7$ CFU/g); ($1.2x10^7$ CFU/g) and ($1.9x10^7$ CFU/g) for total viable (TVC), Staphylococcal, *Salmonella-Shigella* and fungal counts respectively. While Naira denominations from same vendor ranged from ($1.0x10^7$ to $1.7x10^7$ CFU/g); ($5.8x10^6$ to $1.6x10^7$ CFU/g) and ($1.0x10^6$ to $1.4x10^6$ CFU/g) for TVC, Staphylococcal and Fungal counts respectively. There were no observable enterobacteriaceae growths on the currency denominations.

Food vendor 2, jollof rice sample, TVC was (2.0×10^7 CFU/g), Staphylococcal count was (2.0×10^7 CFU/g), *Salmonella-Shigella* was (1.1×10^7 CFU/g) and fungal count was (1.9×10^7 CFU/g) as shown in Table 3. While for the currency denominations obtained from the vendor, TVC ranged from (4.2 to 5.0 x 10^6 CFU/g); Staphylococcal count ranged from (1.2 to 2.0×10^6 CFU/g); *Salmonella-Shigella* count ranged between (1.6×10^6 to 3.2×10^6 CFU/g).

There were no observable coliform growth in currency denominations except 200Naira notes which had an average of (1.5×10^5 CFU/g). However, fungal counts ranged between (5.7 and 7.3×10^5 CFU/g). Total viable count from pounded yam sample was (4.0×10^7 CFU/g), the total Staphylococcal count was (5.1×10^6 CFU/g) the total *Salmonella-Shigella* count was (5.9×10^6 CFU/g), coliform count was (1.0×10^6 CFU/g) while the total fungi count was (8.5×10^5 CFU/g).

Table 2. Microbial load (CFU/g) of food and currency samples from food vendors

Sample	TVC*	Staph	SS[1]	Coliforms	Fungi
FV1					
Ekuru	$1.7\pm0.6^{a}\times10^{8}$	$1.3\pm0.7^{a}\times10^{7}$	$1.2\pm0.2^{a}\times10^{7}$	NG	$1.9\pm0.2^{a}\times10^{7}$
Currency					
Polymer	$1.7\pm0.5^{b}\times10^{7}$	$1.4\pm0.9^{a}\times10^{7}$	NG	NG	$1.1\pm0.4^{b}\times10^{6}$
200Naira	$1.0\pm0.5^{b}\times10^{7}$	$5.8\pm0.4^{a}\times10^{6}$	NG	NG	$1.0\pm0.3^{b}\times10^{6}$
500Naira	$1.2\pm0.8^{b}\times10^{7}$	$1.6\pm0.2^{a}\times10^{7}$	NG	NG	$1.4\pm0.1^{b}\times10^{6}$
FV2					
Jollof rice	$2.0\pm0.3^{a}\times10^{7}$	$2.0\pm0.2^{a}\times10^{7}$	$1.1\pm0.2^{a}\times10^{7}$	NG	$9.3\pm0.2^{a}\times10^{6}$
Currency					
Polymer	$4.5\pm1.2^{b}\times10^{6}$	$1.2\pm0.2^{b}\times10^{6}$	$3.0\pm0.3^{b}\times10^{6}$	NG	$5.7\pm0.6^{b}\times10^{5}$
200Naira	$4.2\pm0.8^{b}\times10^{6}$	$2.0\pm0.3^{b}\times10^{6}$	$1.6\pm0.1^{b}\times10^{6}$	$1.5\pm0.3^{b}\times10^{5}$	$7.4\pm0.3^{b}\times10^{5}$
500Naira	$5.0\pm0.9^{b}\times10^{6}$	$1.7\pm0.5^{b}\times10^{6}$	$1.6\pm0.4^{b}\times10^{6}$	NG	$6.2\pm0.3^{b}\times10^{5}$
FV3					
Pounded yam	$4.6\pm0.5^{a}\times10^{7}$	$5.1\pm0.4^{a}\times10^{6}$	$5.9\pm0.1^{a}\times10^{6}$	$1.0\pm0.8^{a}\times10^{6}$	$8.5\pm0.0^{a}\times10^{5}$
Currency					
100Naira	$9.3\pm0.4^{a}\times10^{6}$	$2.8\pm0.8^{b}\times10^{6}$	$1.7\pm0.8^{b}\times10^{6}$	$1.0\pm0.0^{b}\times10^{4}$	$1.4\pm0.1^{c}\times10^{5}$
200Naira	$3.2\pm0.2^{a}\times10^{7}$	$2.1\pm0.2^{b}\times10^{6}$	$5.6\pm0.8^{b}\times10^{5}$	$9.0\pm0.1^{b}\times10^{4}$	$2.5\pm0.4^{b}\times10^{5}$
500Naira	$2.4\pm0.1^{a}\times10^{7}$	$1.9\pm0.3^{b}\times10^{6}$	$1.3\pm0.8^{b}\times10^{6}$	$5.0\pm0.0^{b}\times10^{3}$	$1.3\pm0.0^{c}\times10^{5}$
FV4					
Suya	$1.3\pm0.4^{a}\times10^{8}$	TNTC	$5.7\pm0.6^{a}\times10^{7}$	$3.1\pm0.6^{a}\times10^{5}$	NG
Currency					
Polymer	$2.8\pm0.6^{b}\times10^{6}$	$1.7\pm0.1\times10^{6}$	NG	$3.7\pm0.2^{b}\times10^{5}$	ND
100Naira	$6.0\pm0.6^{b}\times10^{6}$	TNTC	NG	$6.5\pm0.8^{b}\times10^{4}$	ND
200Naira	$3.0\pm0.4^{b}\times10^{6}$	$1.2\pm0.8\times10^{7}$	$4.9\pm0.1^{b}\times10^{5}$	$6.8\pm0.6^{b}\times10^{5}$	ND

FV= Food vendor; TVC= Total viable count; SS= Salmonella –Shigella; NG= No growth; ND= not determined; TNTC= Too numerous to count; Polymer notes included: 10, 20 and 50 Naira notes. Values are mean ± SD of duplicate; Duncan separation of means with same alphabets are not different (P =< .05) in each column.*

For the change obtained from the vendor after purchase, the counts ranged from (9.3×10^{6} to 2.4×10^{7} CFU/g); (1.9 to 2.8×10^{6} CFU/g); (5.6×10^{5} to 1.7×10^{6} CFU/g); (5.0×10^{3} to 9.0×10^{4} CFU/g) and (1.4×10^{5} to 2.5×10^{5} CFU/g) for TVC, *S*taphylococcal, *Salmonella-Shigella*, Coliform and fungal counts respectively. From food vendor 4, suya sample had total viable count of (1.3×10^{8} CFU/g), staphylococcal count was too numerous to count (TNTC), *Salmonella-Shigella* count was (5.7×10^{7} CFU/g) and total coliform count of (3.1×10^{5} CFU/g) while there was no observable fungal growth For the currency notes obtained, the counts ranged from (2.8 to 6.0×10^{6} CFU/g) for total viable count; (1.7×10^{6} CFU/g to TNTC) for Staphylococcal count; (6.5×10^{4} CFU/g to 6.8×10^{5} CFU/g) for coliform count.

Food handling personnel play important roles in ensuring food safety throughout the food chain of production, processing, storage and distribution. Mishandling and wanton disregard of hygienic measures on the part of the food vendors have been reported to introduce contaminants and pathogens that survive and multiply in sufficient numbers to cause illness in the consumers [23, 24,25,26]. The proper preparation of foods in advance of consumption, exposure to unclean environment, holding at ambient temperature conducive for microbial multiplication coupled with the rich nutrient medium of these foods are factors that increase microbial loads of the samples [27]. Microbial guideline for cooked foods stipulates that "plate counts must be less than 1.0×10^{7}CFU/g for meat; 1.0×10^{4} for plant products [28]. The microbial loads of the food samples were higher than the stipulated, hence, consumption of the foods constitute health risk to consumers. It can be adjudged that the street foods obtained in this study are not bacteriologically fit for consumption. Thus, it calls for improvement in awareness at all levels, especially among personnel working in food establishments, on the possible health risks associated with poor handling of paper currencies while serving/preparing foods. Consequently, the combination of the widespread use of paper currencies and their constant exchange make them a likely agent for various disease transmissions since communicable diseases can spread through contact with fomites [29]. Furthermore, a study revealed that paper currencies can serve as an ideal breeding ground for microorganisms [30]. Pieces of money are in continuous circulation, passing in all environments that constitute a reservoir of various bacteria some of which can survive. Paper currency absorb moisture which encourages the growth of microorganisms on them. Fungi get into money especially from air, soil and where it is kept (for

instance, sacks pockets and socks). Money is contaminated as it circulates from hand to hand [31]. Besides reducing the life span of the notes, some of these fungi have the potential to cause skin, eye and gastrointestinal infections [32, 33], infections of internal organs [34, 35] as well as serious diseases of the respiratory tract [36] in man. In Nigeria, currency notes are often manhandled and mutilated by different categories of people, including traders, churches, beggars and commuter bus conductors [37].

3.2. Identification of microorganisms

A total of 50 isolates were recovered from all the street food and currency denominations.

Isolates from food samples

From food vendors 1 (ekuru and currency samples), 5 and 4 genera were isolated and identified from food and currency denominations (Figs. 1a and 1b). The genera included: *Staphylococcus, Klebsiella, Bacillus, Proteus, Yersinia, Enterobacter and Salmonella.* At least 3 bacteria genera are found in common in the food and change from same vendor.

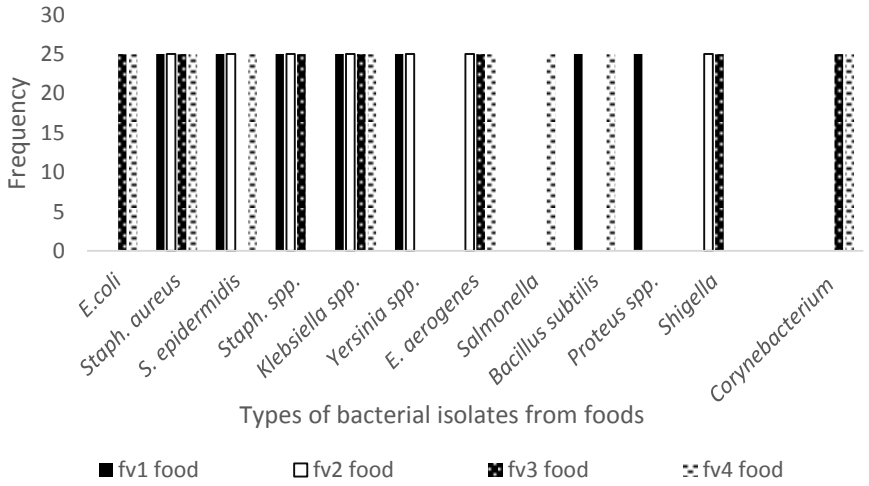

Fig. 1a: Types and frequency of isolation of bacteria from foods (ekuru from food vendor 1; jollof rice from food vendor 2; pounded yam from food vendor 3; and suya from food vendor 4)

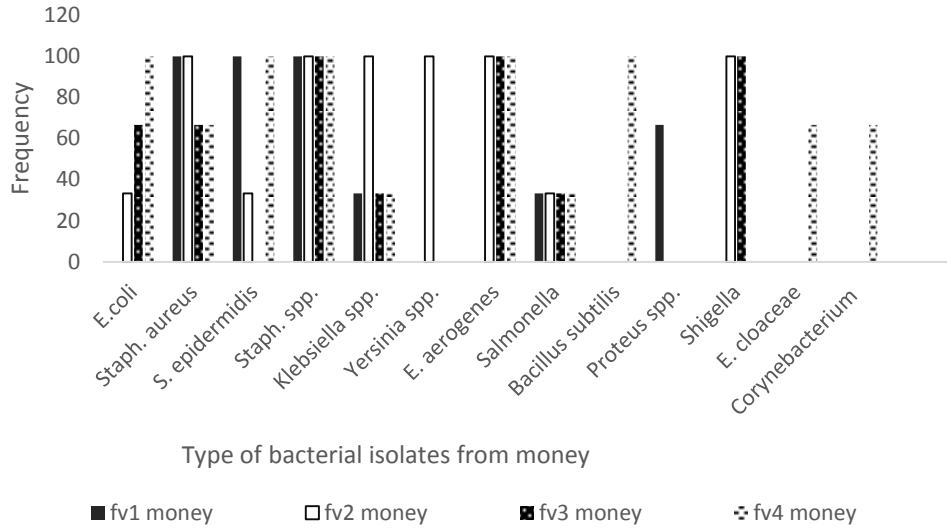

FIG. 1B: Types and frequency of isolation of bacteria from currency denominations received as change from food vendors

From food vendor 2, 5 and 7 genera were identified from food and currency respectively. A total of 7 genera were identified from pounded yam, and 6 from the change obtained from food vendor 3. While 7 and 8 genera from suya and currency were identified respectively. The organisms isolated were observed to be similar to those isolated by Adamu *et al* [38]. *Shigella*, *Klebsiella* spp. *E.coli*, *Staphylococcus aureus* and *Staphylococcus* spp. were isolated in the food samples. According to Asogwa *et al* [39], during preparation and vending, food items like raw meat for 'suya' (roasted pieces of meat attached to a stick) and salad raw materials were prepared using the same knife without in-between washing. After cooking or roasting, foods are continuously exposed to open environment for quick sale and are often invaded by flies [39].

3.3. Antibiotics resistance profile of isolates

Microbial strains showed varied resistance patterns to the different antibiotics screened. C*orynebacterium, E. coli, E. aerogenes, E. cloaceae, Yersinia and Proteus* were multidrug resistant, since multidrug resistance was defined as resistance to ≥3 of the antimicrobial agents tested. it was further observed that bacteria show resistance to ceftazidine (30 µg), cefuroxime (30 µg) and amoxycillin/clavulanate (30 µg) while being susceptible to cefriaxone (CTR), ampicillin (AMP), Nitrofurantoin (NIT) and Oflaxacin (OFL) as presented in Table 3. Furthermore, all Gram negative organisms except *Yersinia* were resistant to Ceftazidime (30 µg). Five of the eight Gram negative were resistant to Ampicillin (10 µg) except *Pseudomonas* (10 mm), *Klebsiella* (10 mm) and *E. coli* (11 mm). The percent resistance for Ceftazidime was (61.5 %), Amoxycillin/Clavulanate (53.8 %), Cefuroxime (30.7 %), Ampicillin (62.5 %) (Fig. 3).

Table 3. Zone inhibition (mm) of antibiotics against foodborne and currency isolates

Isolates (G. Rxn)	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	AMP	NIT	CPR
S.epidermidis (+)	23	29	19	24	21	22	23	30	-	-	-
Corynebact. (+)	R	R	17	27	16	10	14	R	-	-	-
B. subtilis (+)	25	12	26	26	19	21	22	21	-	-	-
S. aureus (+)	13	R	17	16	R	10	18	24	-	-	-
Staph. spp. (+)	15	18	20	26	15	18	26	R	-	-	-
P. aeruginosa (-)	R	10	12	-	-	-	30	20	10	25	26
Klebsiella (-)	R	30	18	-	-	-	23	R	10	34	24
E. coli (-)	R	21	13	-	-	-	R	18	11	24	R
E. aerogenes (-)	R	R	14	-	-	-	18	R	R	25	R

E. cloacae (-)	R	R	24	-	-	-	R	R	R	21	R
Salmonella (-)	R	39	23	-	-	-	31	29	R	39	31
Yersinia (-)	20	23	R	-	-	-	R	R	R	R	R
Proteus (-)	R	21	15	-	-	-	20	R	R	23	21

CAZ= Ceftazidime; CRX= Cefuroxime; GEN= Gentamicin; CTR= Cefriaxone; ERY= Erythromycin; CXC= Cloxicillin; OFL= Ofloxacin; AUG= Amoxycillin/ Clavulanate; CPR= Ciprofloxacin; NIT= Nitrofurantoin; and AMP= Ampicillin; R=Resistant; - = Not tested.

Salmonella, Shigella, E.coli, Yersinia and *Staphylococcus aureus* are public health important organisms that have been demonstrated to pose significant health risks [40]-[41]. Some of the isolates were found to be multidrug resistant particularly, there were higher drugs resistance in the Gram negative organisms as compared to the Gram positive organisms. Salmonellosis and Shigellosis remain a major public health problem across the globe [42]-[44]. Similarly the Centers for Disease Control and Prevention (CDC) estimates that 48 million cases of foodborne illnesses occur in the United States annually [44]. More than 200,000 persons die of food poisoning in Nigeria every year [45]

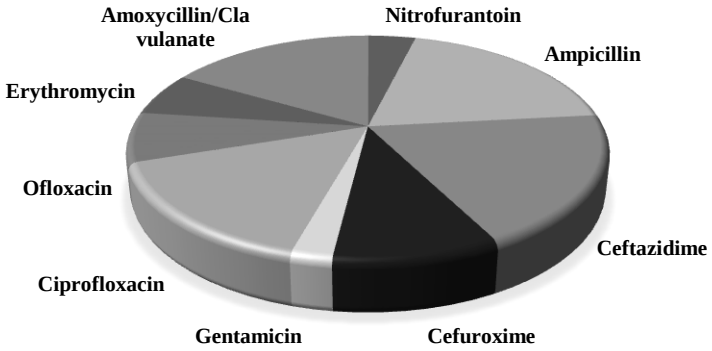

Fig. 3: Antibiotic resistance profile of bacterial isolates from Street vended foods and money

It has been previously reported that indiscriminate use of antibiotics can lead to multidrug resistance [46]. The resistance to antimicrobial agents by bacterial pathogens is a major hindrance to successful therapy and some bacterial strains have been reported to be resistant to most available antimicrobial treatments [47, 48, 49]. In this study, Gram positive organisms were observed to be less resistant compared to Gram negatives, which was also observed by Exner *et al* [50].

4. CONCLUSION

The microbial load of all the foods in this study exceed the stipulated limit for ready-to-eat foods, thereby making consumption of these foods of safety and public health concern. Suya and ekuru food samples had higher microbial loads, followed by pounded yam and jollof rice samples. Some of the organisms isolated from the money samples were also found in the food samples, this could be an indication of cross contamination between money and food. Gram negative isolates were found to have higher drug resistance than the Gram positive isolates. Also, some of the organisms were found to be multidrug resistant. Most of the isolated organisms in this study were resistant to Ceftazidime (30µg) while *Yersinia* was resistant to most of the drugs.

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